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Abstract

Various chemical, physical, and structural analyses were conducted on cell-wall materials isolated from the green algae *Bryopsis plumosa*, *Cladophora glomerata*, and *Chara globularis*. The potential of each algal material to modify paper structural and mechanical properties was considered by comparing their characteristics to those of a softwood sulfite pulp.

Cell-wall materials, isolated by boiling algal biomass in dilute alkali, formed 19-31% of original thallus weights. Cell walls of each alga remained intact upon recovery and maintained lengths of up to several centimeters, but these were cut later to match those of the softwood tracheids. Although units of cell-wall material from each alga displayed large variations in collapsed width, all averages were substantially larger than those for the tracheids. Processed cell walls from each alga had thicknesses that were *ca.* 25-50% of those in the tracheids. Cellulose was the main structural polysaccharide in the *Cladophora* and *Chara* materials; xylan predominated in *Bryopsis*. Several analyses demonstrated the cellulose crystallinities of *Cladophora* and *Chara* materials to be somewhat greater and smaller, respectively, than that of the softwood pulp.

Polarized-light analysis supported previous descriptions of crossed-helical microfibril arrays in *Cladophora* by showing a net angle of microfibril orientation that was nearly parallel to the longitudinal cell axis. A net orientation could not be detected in the *Bryopsis* material, corresponding to reports that describe a random placement of short, rod-like microfibrils within the plane of these cell walls. Only a faint indication of a near-transverse net angle was detected in *Chara* materials, suggesting that microfibrils are distributed evenly between the longitudinal and transverse axes of the cell wall. This finding could be attributed to previously reported, passive redistributions of initially transverse microfibrils during cell elongation. However, helicoidal constructions detected here by transmission electron microscopy also might explain such distributions.

The relatively thin cell walls and large collapsed widths associated with each algal material were considered characteristics likely to impact handsheet properties, because of their potential to increase contact between surfaces in the network. Certain factors, such as degree of cellulose crystallinity or net angle of microfibril orientation in helical arrays, have known effects upon strengths of softwood tracheids, though their influence upon the performance of nonwoody materials remains uncertain. The occurrence of cross-walls in the *Cladophora* materials, the randomly oriented, xylanaceous microfibrils of *Bryopsis*, and the easily dissociated lamellae and helicoidal constructions in the *Chara* cell walls are examples of other attributes that have yet to be incorporated into models of paper structure and mechanics.

Introduction

The algae are a diverse collection of eukaryotic, photosynthesizing organisms. Algae do not constitute a single, closely related taxonomic grouping and do not represent a natural alliance of organisms. Most modern biologists distinguish the algae from plants and categorize them among six different divisions within the Kingdom Protista. Like the bryophytes (mosses) and vascular plants of the Kingdom Plantae, algae have photosynthetic systems based upon chlorophyll *a*. Unlike plants, however, most algae do not develop as embryos that are surrounded by a protective covering produced by the parent. Algae do not possess vascular systems. Also, the reproductive structures of algae differ in that they are generally single-celled and are not associated with sterile tissues (Raven *et al.*, 1986).

Some algae may exist as microscopic unicells; others may be organized into multicellular bodies (thalli) and take the form of simple filaments or more-massive, complex structures that can grow to lengths of over 50 m. Algae chiefly inhabit aquatic environments, both freshwater and marine, but they also may be found living in soil, exploiting various subaerial habitats, or maintaining symbiotic relationships within the tissues of both plants and animals. Algae are important primary producers. For example, photosynthesis in the oceans, which is dominated almost exclusively by algae, provides more than 33% of total global carbon fixation (Whittaker, 1975).

Algal cell walls, like those of plants, most frequently are constructed of polysaccharides that are organized into crystalline microfibrils embedded in and surrounded by a noncrystalline matrix. Some algae incorporate minerals into their cell walls. Algal cell-wall microfibrils often are composed of cellulose, but also may consist of xylans or mannans. The chemistry, physical structure, and biological formation of algal cell walls have been examined extensively over the past three decades (Roelofsen, 1959; Parker, 1970; Haug, 1974; Mackie & Preston, 1974; Mizuta & Okuda, 1987).

Although historical records indicate that the Chinese utilized "seaweed" for papermaking as early as the 4th century A.D. (Tsien, 1973), very few studies have considered how the mechanical properties of algal materials might affect their potential use in papermaking processes. In a survey of several marine algae, Kiran *et al.* (1980) reported that certain strength properties of pulp and handsheets prepared from the green alga *Chaetomorpha* were notably larger than those from other tested species and attributed these results to a comparatively high (36.5%) cellulose content. Although the low levels of fibrillar polysaccharides recoverable from many other algae (Preston, 1974; Painter, 1983) probably have discouraged more-extensive investigations in these areas, some workers nevertheless have developed successful patents for algal-pulp preparations (Ogaki *et al.*, 1979; Ogaki, 1980).

As part of an ongoing investigation of algal fibrillar, or structural, polysaccharides, we have sought to evaluate the potential utility of such substances as modifiers of paper properties. In the present study, various chemical, physical, and structural properties are described for cell-wall materials isolated from algae in three different orders of the Division Chlorophyta (the green algae). A species of *Bryopsis* was selected for testing because members of this genus are known to have cell walls composed primarily of a β-1,3-linked

xylan. The other two specimens were chosen to study contrasting forms of cellulose: the microfibrillar cellulose isolated from *Cladophora* cell walls is highly crystalline, whereas that isolated from *Chara*, representing a taxonomic group from which land plants most likely descended, has a crystallinity similar to that of wood pulp. A softwood pulp was included in these characterizations and also was employed in the second stage of this study (Hackney *et al.*, 1995) to provide controls for comparisons of handsheet structural and mechanical properties.

Materials and Methods

Pulped softwood fibers and the cell-wall materials from three green algae were characterized. The softwood pulp ("Puget Prime") was a bleached sulfite pulp dominated by tracheids from various gymnosperms, chiefly white fir, Douglas fir, spruce, and hemlock. Three algae from the Division Chlorophyta were examined: *Bryopsis plumosa* (Hudson) C. Agardh, a coenocytic, marine species in the Order Caulerpales; *Cladophora glomerata* (L.) Kutz., a multicellular, freshwater member of the Order Cladophorales; and *Chara globularis* Thuill., a multicellular, freshwater, calcified alga of the Order Charales. Samples of *Bryopsis* were collected from the central display tank at the Seattle Aquarium in Washington. *Cladophora* was cultured in our laboratory in defined medium (Graham *et al.*, 1982). *Chara* was collected from alkaline lakes in central Wisconsin. Each type of alga was treated chemically to convert it to a form that could be used in handsheet formation. The softwood pulp was obtained commercially and did not require such preparation.

Preparation of Algal Cell-Wall Materials. Algal thalli were preserved in 100% methanol. Prior to chemical treatment, preserved biomass was air-dried to stable damp weights. Sub-samples of this material then were oven-dried to determine moisture content. These two weights were used to calculate the portion of alcohol-preserved weight that was recovered as structural polysaccharides following chemical treatment.

The *Chara* thalli first were decalcified by soaking for several hours in 1% HCl. Lipids and photosynthetic pigments then were removed from each of the three algae by two consecutive, 24-h soxhlet extractions, using 100% methanol followed by 2:1 chloroform:methanol. Afterwards, extracted material was rinsed thoroughly with methanol, followed by distilled water. Biomass next was given three consecutive, 2-h boils under N_2 atmosphere and reflux in a solution of 0.25-*M* NaOH, to which *ca.* 0.2 g/L $NaBH_4$ was added to form complexes with the ends of cellulose chains and, thus, minimize degradation by the alkaline peeling reaction (White & Kennedy, 1988). These boils in alkali removed the majority of disordered polysaccharides from the cell walls, save for those polymer chains that may have been inserted within the ordered lattice of the crystalline regions or bound to the surface of these crystallites with particular strength.

Following a thorough rinsing with distilled water, the algal materials were soaked for 24 h in a 0.1-*M* solution of $ClNaO_2$ dissolved in 1% glacial acetic acid. This bleaching step converted or removed any residual chromophores. Then, following a distilled-water rinse, the materials were given three 1-h soakings in a 0.1% solution of the chelator diethylenetriamine pentaacetic acid (DTPA) to remove minerals. After a final distilled-

water rinse, the materials were freeze-dried to prevent bonding between cell walls (hornification) and to ensure free-dispersion in water during handsheet formation.

This series of treatments effectively dissolved less-ordered cell-wall components, isolating the structural polysaccharides (xylan in *Bryopsis*, cellulose in *Cladophora* and *Chara*) within the algal cell walls. Essentially the same processes had been accomplished during the commercial sulfite-pulping and bleaching of the softwood, which removed most of the lignin from within and between cell walls, separated the individual tracheids, and dissolved lower-molecular-weight materials that were complexed with the cellulose. (Lignin did not occur in the algae studied here.)

Because the lengths of processed algal cell-wall materials ranged to several centimeters in some instances and would present difficulties for handsheet formation, freeze-dried algal materials were cut by scalpel to *ca.* 4 mm, a length chosen to match that of typical softwood tracheids. Because neither the terms "fiber" nor "filament" may be employed properly as a common label for all four forms of biomass examined in this study, individual lengths of cell wall prepared here are referred to collectively as "units" of cell-wall material.

Sugar Analyses. A recently modified technique of wood-sugar analyses (Pettersen & Schwandt, 1991) was utilized to identify the neutral sugar monomers within duplicate samples of each type of cell-wall material. Dilute-acid (H_2SO_4) hydrolyzates of the prepared materials first were injected directly onto an anion-exchange liquid-chromatography column without prior neutralization or concentration and then were separated using a 0.25-m*M* NaOH eluent. The post-column stream was directed through a pulsed-amperometric detector cell, where amounts of each monomer were measured as increases in oxidative current passing through a gold electrode. Concentrations were quantified by comparison to standards of individual monomers.

X-ray Diffraction Analyses. X-ray powder diffraction patterns were collected using disks that were pressed from samples of each type of cell-wall material. Patterns were recorded on a Philips model APD-3720 diffractometer, employing Cu-K_α radiation in the reflection mode of diffraction. The diffractometer was equipped with a graphite monochromator and utilized a sampling interval of 0.02° two-theta, a sampling time of 1 s per interval, and a scanning range of 4-40° two-theta.

Crystallinity indices of the cellulosic materials were calculated from these diffraction patterns using the "CrI" measure of Ahtee *et al.* (1980). This index compares the height of the 020 peak to that of the lowest point on the pattern between the 110/110 and 020 peaks.

Cell-Wall Densities. The densities of each type of cell-wall material were measured on a density-gradient column following the techniques of Harkin and Obst (1974a,b). Twenty separate fractions of the column were prepared by blending different ratios of two organic liquids (CCl_4 and toluene), establishing densities that were expected to encompass those of the four samples. After these liquid fractions were loaded sequentially onto the column and carefully mixed to set up a linear gradient, the column was calibrated with glass beads of known density. Triplicate samples of cell-wall material were soaked thoroughly in an aliquot of the least-dense fraction and then were introduced separately to

the top of the column. After settling within the column for 24 h, sample positions were noted for density calculations. Results were compared to the recorded density of a microcrystalline-cellulose standard (Avicel™).

Structural Characteristics. Samples of both preserved, nontreated algal thalli and processed cell-wall materials were subjected to scanning electron microscopy (S.E.M.). Surfaces were shadowed with gold-palladium and examined with a JEOL JSM-35C scanning electron microscope to improve visualization of certain structural characteristics.

For transmission electron microscopy (T.E.M.) analysis, *Chara* specimens were collected and maintained in soil water on a sunny window sill for approximately one year before newly grown shoot tips, consisting of noncorticated internodal cells, were harvested. These tips were soaked in a series of fixatives that had glutaraldehyde concentrations gradually increased to 2%. Shoot tips were then rinsed in a phosphate buffer, post-fixed in 1% osmium tetroxide, and re-rinsed in phosphate buffer. They next were dehydrated in a graded ethanol series, and then embedded in Spurr's low-viscosity plastic (Polysciences, Inc.). Sections were collected on 200-mesh copper grids, stained with lead citrate, and examined and photographed with a JEOL JEM-1200-EX transmission electron microscope.

After samples of each algal material were mounted on glass slides, cell-wall thicknesses and collapsed widths were measured by light microscopy. For both characteristics, a counting grid was employed to keep track of 200 measurements in multiple fields of view. For cell-wall thicknesses, materials were stained with Graff's "C" stain and measured with an objective-mounted micrometer. Widths of collapsed units were measured electronically following the technique of Quirk (1981). Materials were mounted in a 1:1 glycerine:ethanol mixture, their microscopic images were projected onto a viewing screen, and a probe, attached to a sonic-digitizing unit, was employed to measure distances across these screen images. Using a computer, the data were recorded as x-y coordinate values, digitized, and then converted to appropriate units of measure based upon the degree of magnification used with each sample.

The relatively thin cell walls of each alga allowed direct examinations by polarized-light microscopy for evidence of microfibril orientation (Preston, 1974; Richmond, 1983). Algal thalli were sectioned longitudinally so as to prepare single layers of cell-wall material. Following a Graff's "C" stain, these materials then were mounted under coverslips and viewed in plane-polarized light at 400X magnification. Slide preparations were rotated on the stage to any point of light extinction, and the angular difference between this point and the longitudinal axis of the cell determined the net fibril angle, or that angle of microfibril orientation predominating within the cell wall. Both the primary and any detectable secondary fibril angles, of less intensity than the first, were recorded. Such analysis of the softwood tracheids was prevented by the thickness of their cell walls.

Results

Based upon damp weights at the beginning of chemical treatment, that portion of total biomass recovered as structural polysaccharides was 19.3, 27.4, and 31.3% for *Bryopsis*, *Cladophora*, and *Chara*, respectively. Yields for softwoods subjected to sulfite-pulping typically are 43-48% (F.P.L., 1953).

Cells were emptied completely of cytoplasm during chemical treatments of the algal thalli. Although nonstructural polysaccharides were solubilized during these procedures, the cell walls of the materials remained intact. In the case of *Chara*, the treatments separated thalli into walls that formerly delimited individual cells. Prior to chemical treatment, the *Chara* thallus was constructed of a series of elongated, cylindrical cells arranged in an end-to-end fashion to form a main axis and multiple branches. Each of these internodal cells in turn is encircled by a layer of *ca.* 12-16 corticating cells. Though also cylindrical and generally of the same length as internodal cells, these corticating cells have markedly smaller diameters and calcified surfaces (Fig. 1). The walls of individual *Chara* cells that were isolated by chemical treatment possessed slightly flared ends and displayed a wide variation in diameters (Fig. 2).

In the case of *Bryopsis* and *Cladophora*, recovered cell walls tended to preserve the gross morphologies of the intact, untreated thalli. The *Cladophora* thallus takes the form of a branched, uniseriate filament of cylindrical cells. In such a filament, adjacent daughter cells remain connected in an end-to-end fashion, sharing the cross-wall that formed during their division. These daughter cells also retain portions of the cylindrical walls of their parent cell, laying down additional lamellae upon the inner surfaces of the walls as they are stretched and partially torn during cell growth. Consequently, parts of a parent cell wall may provide the outer lamellae for several adjacent daughter cells within the filament (Preston, 1974), establishing a system of protective, overlapping sheaths that helped to prevent the separation of individual cells during chemical treatment. The diameter of the chemically treated *Cladophora* thallus was constricted at the position of each cross-wall (Figs. 3 & 4).

As a coenocytic alga, *Bryopsis* differs from both the other algae in this study by having a vegetative growth pattern that involves divisions of nuclei without subsequent divisions of the cytoplasm. Thus, the *Bryopsis* thallus consists of a single, multinucleated cell. *Bryopsis* somewhat resembles the branched filament of *Cladophora* when viewed macroscopically, but neither its axis nor branches are partitioned by cross-walls. When recovered from chemical treatment, the cell-wall material from this alga consisted of long, hollow tubes bearing shorter, tubular branches, though considerable separation of these two components occurred during freeze-drying (Fig. 5).

Of the three forms of algal cell-wall material, then, only the units derived from *Chara* thalli were similar to softwood tracheids (Fig. 6) in consisting of walls from individual cells. Also, as a result of being hand-cut to a standard length, each type of algal unit frequently possessed relatively squared ends as opposed to the tapered ends of tracheids. Cell-wall thicknesses and the widths of collapsed algal units (Table 1) differed considerably from those of the softwood pulp. Only the thickest walls of the *Bryopsis*

units lay within the range normally encountered for softwood tracheids, and the majority of values for all three types of algal unit were less than 2.4 μm, the lower limit of detection for this analysis (contrast Figs. 7 & 8). The widths of *Chara* units varied between the internodal and corticating cells, and the flattened widths of *Cladophora* units varied with the portion of the filament that was selected for measurement. In addition, the widths and lengths of individual cells in *Chara* and *Cladophora* and the widths of the coenocytic axes of *Bryopsis* decreased from the older, more-basal portions of the thalli to the younger tissues in the apices. Such variations increased standard deviations associated with the flattened algal-unit widths. Because most tracheids from softwood tissues have matured and display comparatively consistent dimensions at the time of harvest, variations in the widths of these units most probably reflect inclusion within the pulp of cells from multiple species.

S.E.M. confirmed that cell walls of each alga were constructed of multiple, thin lamellae having uniform thicknesses. The lamellae of these walls did not appear to be differentiated structurally from one another, as is observed, for example, in the wall layers of softwood tracheids. However, upon recovery from chemical treatment and cutting to the standard length, walls from the larger, more-mature internodal cells of *Chara* thalli often were separated into 3-4 layers of multiple lamellae (Fig. 9). Such separations appeared to occur randomly within these treated and cut cell walls and could be promoted easily by teasing the walls with a metal probe.

T.E.M. micrographs of *Chara* material provided evidence of helicoidal constructions within cell walls. In obliquely cut sections of the shoot-tip internodal cells, 8-10 layers of arced striations often were detected in a zone that lay near the middle of the secondary cell wall (Fig. 10). Such patterns generally are interpreted as indicating the presence of helicoidal patterns of microfibril orientation.

The *Chara* cell walls displayed little evidence of oriented microfibrils; only a weak indication of net fibril angle could be detected at a nearly transverse orientation, *i.e.*, at just less than 90° to either side of the longitudinal cell axis. In contrast, a strong extinction of light was detected at about 1° from longitudinal in the walls of *Cladophora*. No extinction, however slight, could be observed during examinations of *Bryopsis* cell walls. Although not measured in this study, the net microfibril orientations of the softwood tracheids employed here are reported to lie most commonly at 15-30° and, infrequently, below 10° from the longitudinal axis (Mark, 1967).

The results of the sugar analyses (Table 2) confirmed that glucose was the monosaccharide predominating in cell walls of softwood tracheids, *Cladophora*, and *Chara*. Xylose was the monosaccharide most abundant in *Bryopsis*. The total mass that could be accounted for by monosaccharides was *ca.* 95% for *Cladophora*, which may indicate the inclusion of noncarbohydrates within these cell walls. This value ranged from 100-106% for the other materials, demonstrating some degree of over-estimation. Among the cellulosic materials, nonglucose monosaccharides accounted for about twice as much of the cell wall of *Chara* (21.9%) as of either *Cladophora* or the softwood pulp (9.3 and 11.6%, respectively).

Peak positions of the softwood-pulp, *Cladophora*, and *Chara* x-ray diffractograms (Fig. 11) corresponded to those of cellulose standards (Alexander, 1969; Okano *et al.*, 1989).

The pattern for the *Bryopsis* material duplicated that of previously examined samples of algal β-1,3-xylan (Hackney, personal observations). A visual assessment of peak widths suggests that the cellulose of *Cladophora* is more crystalline than that of *Chara* or the softwood pulp, which appear to share approximately equal crystallinities. These observations were supported generally by the unitless crystallinity indices of these cellulosic materials: softwood pulp = 0.89, *Cladophora* = 0.92, and *Chara* = 0.80.

Replicate samples of each type of cell-wall material settled to consistent positions on the density-gradient column. Values for cellulosic materials (Table 3) showed that densities of the treated *Cladophora* and *Chara* samples were somewhat larger and smaller, respectively, than the nearly identical values recorded for the softwood pulp and microcrystalline-cellulose standard. The *Bryopsis* cell-wall material was considerably less dense than the cellulosic materials.

Discussion

The different characteristics analyzed here distinguished the algal cell-wall units from the softwood tracheids to varying degrees. Although pronounced differences in physical and chemical properties could be recognized only for the xylanaceous *Bryopsis* material, the structural properties for all three types of algal unit were consistently distinct from those recorded for the pulp. The attributes described for each type of unit in the present analyses become of interest for their potential to affect, in turn, the structural and mechanical properties of test handsheets prepared in the second part of this study (Hackney *et al.*, 1995).

This work has suggested that chemical and physical differences between the pulped softwood tracheids and the materials isolated from the two cellulosic algae may be related largely to variations in cellulose crystallinity. The calculations of indices from the diffraction patterns provided direct measurements of cellulose crystallinities, which are known also to correlate directly with densities of cellulosic materials (Ward, 1950). Measurements of the percentage of nonglucose monomers merely indicated the potential of noncellulosic polysaccharides to disrupt the crystalline lattice and, thus, decrease crystallinity. Measurements from the density-gradient column provided the total density of all components within the intact materials. However, each of these tests ranked the cellulose in the *Cladophora* cell walls as most crystalline, followed by that in the softwood pulp, with the cellulose in *Chara* the least crystalline. Although placement of xylan within any numerical scale relating to cellulose is not possible, x-ray diffraction and sugar analysis both provided indications that crystallinity of the *Bryopsis* material lies somewhat below those of the cellulosic cell walls (broader peak widths and smaller proportional contribution by xylose, respectively). Measurements from the density-gradient column also indicated that the total density of *bryopsis* lay below those of the cellulose materials.

As indicators of the crystallinity of each algal structural polysaccharide, such measurements also may predict strength properties for individual units. Tests on cellulosic fibers have demonstrated that crystallinity correlates directly with both tensile strength and Young's modulus, and varies inversely with extensibility (Howsmon & Sisson, 1954;

Salmén & de Ruvo, 1985). However, a decreased crystallinity of individual pulp fibers actually may improve certain paper strength properties. As Page (1983) has argued, disordered regions in fibers are viscoelastic and, thus, more capable of absorbing mechanical stress than crystalline elements. When formed into a network, fibers having greater proportions of disordered regions prove more flexible, require larger strains to separate, and, thus, lend greater tearing strength to a handsheet. There is an upper limit to the improvements gained by increases in the disordered content of pulp fibers, however, and it is uncertain whether such improvements could be expected in handsheets formed from nonwoody or noncellulosic cell-wall materials.

The cell walls of each alga had thicknesses that ranged generally from one-half to less than one-quarter of those for the softwood tracheids. Thin-walled materials tend to collapse more thoroughly during handsheet formation (Page, 1967). Notably large width values in this study (Table 1) indicate an efficient flattening by each type of algal unit when mounted under glass coverslips. Such flattening would be expected to affect handsheet structures by decreasing calipers and increasing densities of prepared sheets. Large collapsed widths in turn could affect paper mechanical properties, because an increased width of units traversing the planar dimension of handsheets also would increase the relative area of surface contact and the degree of subsequent bonding between individual units. Alexander and Marton (1968) observed that the bonded area between tracheids increased following mechanical treatments (beating or wet-pressing) of softwood kraft pulps, improving the in-plane strength properties of prepared handsheets. The authors demonstrated that, under the particular conditions of their study, inter-tracheid bonding played an even greater role in determining sheet strengths than did the tensile properties of individual tracheids. An increased flattening during formation would be expected to improve not only bonding between algal units, but also bonding across lumena of individual units, possibly enhancing strength properties in the thickness direction of the handsheets.

The periodic occurrence of cross-walls within the *Cladophora* materials may provide considerable potential for influencing handsheet structural or mechanical properties. Separated by distances of *ca.* 150-250 μm, these cross-walls would provide a transverse reinforcement of individual units that is absent in both the hollow, tapered tracheids and the hollow, open-ended tubes formed by *Bryopsis* and *Chara* cell walls. The apparent failure of these cross-walls to prevent a thorough collapse of the *Cladophora* units (Fig. 4) suggests that handsheet strengths would be improved to at least some degree by such reinforcement. Alternatively, the occurrence of each cross-wall might be expected to lower tensile strength, because the constriction of the units at these sites (Table 1) would concentrate stresses into a decreased cross-sectional area (Beer & Johnston, 1981). Should these constrictions provide a significant source of tensile failure, they would compare to the nodes, microcompressions, crimps, and other structural irregularities that lower elastic moduli of wood-pulp fibers (Page *et al.*, 1972, 1977, 1979; Page & Seth, 1980).

Although each of these algae has been demonstrated previously to share microfibrillar cell-wall constructions, a clearly discernible net angle of orientation could be detected here only for the *Cladophora* units. We observed a single, low net fibril angle for *Cladophora glomerata*. However, Frei and Preston (1961a,b) showed that cell walls of other species in this genus were constructed so that lamellae having parallel microfibrils

approximately transverse to the cell axis alternated with lamellae having microfibrils nearly longitudinal to this axis. This shift between orientations that differed by nearly 90° was repeated across the thickness of cell walls, though in some instances a third lamella, having microfibrils that also were oriented near the longitudinal axis, was included within the sequence. Such a "crossed-helical" arrangement, in which microfibrils of each orientation form counter-directional spirals within the cell wall, also is observed in the S_1 and S_3 cell-wall layers of many softwood tracheids (Preston, 1974). The low net angle of orientation that predominates in the *Cladophora* cell walls of this study, considerably smaller than that of the softwood tracheids, could impact the tensile strength of individual units. For a variety of single cells having such helically arranged microfibrils, a decreased net angle of orientation has been correlated with increased values of longitudinal elastic stiffness, longitudinal elastic modulus, and standard tensile strength (Alexander *et al.*, 1968; Mark & Gillis, 1973; Page *et al.*, 1977; Salmén & de Ruvo, 1985), but with diminished values of percentage stretch and tensile energy absorption (Morton & Hearle, 1975; Wainwright *et al.*, 1976).

The failure to detect a net fibril angle on the *Bryopsis* material may support descriptions of microfibrils in this alga as being short and rod-like and as displaying a generally random orientation within the plane of the cell wall (Frei & Preston, 1964; Preston, 1974). Such randomness would reinforce the cell wall in a manner that differs from helices formed by parallel microfibrils, in effect lending resistance to stress that impinges from any direction within the plane. However, the total strength available to counter any single stress is reduced accordingly, and Krenchel (1964) has calculated that the theoretical efficiency of reinforcement for such an arrangement drops to *ca.* 3/8 of that which would be provided by microfibrils arranged uniformly in the direction of the stress. In contrast, a uniform alignment provides maximal strength when oriented parallel to the direction of stress, but contributes virtually nothing should the stress shift 90° within the plane. The conformation of the linear xylan that constitutes *Bryopsis* microfibrils also may affect indirectly the strength of both the cell-wall materials and the subsequently formed handsheets. The β-1,3-linkage of this xylan causes it to coil helically. Atkins *et al.* (1969) have concluded that the polysaccharide forms a triple helix, with the helical axis parallel to the microfibril axis. Frei and Preston (1964) and Preston (1974) have observed that microfibrils of the *Bryopsis* cell wall adhere strongly to one another, never forming a loose fringe at the edge of a torn lamella, and are difficult to separate in alkali-treated material. The authors propose that such adherence is established when loose coils of the xylan polymer protrude from microfibril surfaces, promoting entanglements between microfibrils and between these structures and the surrounding matrix polysaccharides. In certain other closely related, xylanaceous algae, electron micrographs reveal that such entanglements may develop into discrete cross-bodies that link microfibrils (Preston, 1974). Thus, these types of connections establish three-dimensional latticeworks within the cell wall, providing reinforcement both within and perpendicular to the planar dimension. By more effectively distributing stresses imposed across the thickness of the cell wall, such construction approaches that of feltwork composites, which are recognized to resist shearing forces far more effectively than two-dimensional, helical arrays of microfibrils (Wainwright *et al.*, 1976).

The weak indication of a near-transverse net fibril orientation in the *Chara* material is more difficult to relate to the literature, because a valid understanding of cell-wall construction in charophycean algae has begun to emerge only recently. By itself, this finding might suggest that *Chara* shares a cell-wall construction that once was ascribed frequently to various species of *Nitella*, a closely related charophyte. During polarized-light analyses, mature *Nitella* cell walls have been described as consisting mostly of randomly oriented microfibrils (Green, 1960; Gertel & Green, 1977), whereas walls of young, actively growing cells were arranged transversely (Green, 1958; Richmond, 1983). These studies concluded that microfibrils are deposited in the *Nitella* cell wall with a transverse orientation and then are reoriented passively by longitudinal expansion of the cell during growth. Such action, described by the multinet growth hypothesis (Roelofsen & Houwink, 1953), would distribute most microfibrils evenly between transverse and longitudinal orientations, leaving only those most-recently deposited in the inner-most lamellae to provide a slight net tendency towards the transverse during polarized-light analysis. The helical microfibril arrays detected here in *Cladophora* also could have been reoriented in this manner during growth, decreasing their original angle of orientation.

However, our identification of arced striations in portions of the *Chara* cell wall would place this alga among the variety of photosynthetic organisms that have cell walls formed in part by helicoidal constructions. Helicoidal constructions are made of successive layers of parallel, helically arranged microfibrils. Between successive lamellae, these microfibrils undergo a small, regular, and progressive shift in helical pitch (Neville, 1985; Neville & Levy, 1985; Satiat-Jeunemaitre, 1992). It is crucial to this discussion to note that a **helicoidal** cell-wall construction is not synonymous with the simpler **helical** arrangement of microfibrils within the walls of the softwood tracheids or *Cladophora*: the two terminologies often are confused. Helicoids have been observed in the walls of oospores and vegetative cells from various other species of *Chara* and *Nitella* (Neville *et al.*, 1976; Neville & Levy, 1984; Leitch, 1989; Nyberg & Saranpää, 1989; Morrison *et al.*, 1993). Most researchers believe helicoids primarily are self-assembled outside the plasma membrane, where high concentrations of hemicelluloses form cholesteric liquid-crystal phases that position the insoluble cellulose microfibrils into helicoidal arrangements (Vian *et al.*, 1986; Neville, 1988; Roland *et al.*, 1989; Vian & Reis, 1991; Reis *et al.*, 1992). Yet, the variations and flexibilities observed in helicoid formation suggest that cells maintain considerable control over the process (Satiat-Jeunemaitre, 1991, 1992). Because microtubules frequently lie parallel to microfibrils during formation of crossed-helical cell walls, they are hypothesized to orient microfibrils by directing movements of cellulose-synthase complexes in the plasma membrane and, thus, to have at least the capability of playing a role in helicoid formation (Roberts *et al.*, 1985). However, helicoid formation fails to correlate with microtubule placements in numerous organisms (Satiat-Jeunemaitre, 1992) and may even be disrupted when a chemical inhibitor of microtubules is withheld from cultures of the filamentous green alga *Chamaedoris orientalis* (Mizuta *et al.*, 1989). Satiat-Jeunemaitre (1991) considers that microtubules likely affect helicoid construction indirectly by mediating the transport of hemicelluloses within Golgi vesicles to the plasma membrane, thereby controlling the balance between hemicelluloses and cellulose.

Owing to the regular orientation of microfibrils in all directions within the plane of

the cell wall, a completely helicoidal arrangement of microfibrils would appear isotropic and indistinguishable from totally random constructions during polarized-light analyses (Levy, 1991). However, based upon our examinations of *Chara* shoot-tip internodal cells, helicoidal constructions may have been too thin to dominate birefringence in this analysis, allowing other, nonhelicoidal zones of microfibrils to provide the weak indication of a net transverse orientation. The limited zone of helicoidal construction detected in this preliminary examination must also be a factor when considering potential influences upon structural and mechanical properties. For this *Chara* material, it is unknown to what extent helicoids contribute to the structure of walls in cells at varied stages of growth or in corticating cells. Thus, it is uncertain whether enough helicoidal structure would be present to affect properties of either the units of cell-wall material or of subsequently formed handsheets. It may prove difficult ultimately to identify helicoidal structures as a stable characteristic of any particular species. Vian *et al.* (1993) have emphasized the transient nature of helicoidal orders within primary walls and their balance between structuring forces and growth-related degradations of order. Cell elongation disrupts helicoidal constructions, causing a progressive thinning of arced striations toward the outer surface of a wall (Erickson, 1980; Levy, 1991). After cessation of growth, the walls of mature internodes in *Nitella translucens* frequently display helicoidal constructions on their inner surfaces (Levy, 1991; Morrison *et al.*, 1993). However, such zones are much-less developed in slowly expanding cells and are completely absent under conditions of rapid growth (Morrison *et al.*, 1993).

Vian *et al.* (1986) have associated high levels of glucuronoxylans with helicoidal zones in the cell walls of Linden wood fibers, and have produced pronounced gaps in these zones by extracting this hemicellulose with alkali. This suggests that an extraction of helicoid-associated hemicelluloses from mature *Chara* internodals contributed to the separation of the cell walls into discrete layers of lamellae. Whatever their origin, these delaminations seem capable of affecting handsheet mechanical properties should they persist to any significant degree after formation and wet-pressing. In particular, decreased strength might be expected under tensile stress in the thickness direction or under shear stress in either the planar or thickness direction of the handsheet. Thus, declines in these strength properties would counter any gains to be derived from cellulose that persisted in helicoidal structures following chemical treatment. The small angular differences between microfibril orientations in helicoids essentially strengthen cell walls in all planar directions and, when compared to simple helical configurations, lend a more effective resistance to shear forces that promote delamination or axial splitting of fibers (Wainwright *et al.*, 1976; Neville & Levy, 1984). Various pines, cedars, spruces, and firs are reported to intersperse helicoids within their helical microfibril arrays, assembling them as thin transition zones between the S_1, S_2, and S_3 layers in walls of tracheids and other cells (Parameswaran & Liese, 1981, 1982; Neville & Levy, 1984). Because tracheids from these same gymnosperms often are included in softwood pulps, it is conceivable that helicoidal constructions have been a traditional, though unrecognized, source of strength enhancement for many types of paper products. However, the effectiveness of limited zones of helicoidal construction remains in question, and this uncertainty also must temper any consideration of impact by *Chara* helicoids in the present study.

The various questions raised by observations in this phase of the study emphasize the need to recognize each of these algae as nontraditional sources of papermaking materials. In these analyses, each type of processed algal unit displayed certain features that contrast with those of softwood tracheids, whose helical arrays of cellulose microfibrils traditionally have formed the basis for theories of paper structure and mechanics. These features potentially could impact several different levels of handsheet organization, providing variations in the conformation and crystallinity of structural polysaccharides, in the size, shape, and orientation of microfibrils within the cell wall, in the thickness, diameter, and integrity of cell walls, and in the extent and manner of bonding between units of cell-wall material. The question of whether any of these features enable the algal materials to modify paper properties significantly is addressed in Part 2 (Hackney *et al.*, 1995).

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Table 1. Thicknesses of cell walls and widths of collapsed units for softwood pulp and three types of algal material. Thicknesses listed as ranges of measured values, widths presented as means followed parenthetically by standard deviations. Widths subdivided into values for *Chara* internodal and corticating cells and into measurements taken at mid-cell and at cross-walls of *Cladophora* units.

CELL-WALL MATERIAL	THICKNESSES (μm) of CELL WALLS	WIDTHS (μm) of FLATTENED UNITS
Softwood Pulp	4.0 - 7.0	22.6 (±7.9)
Chara globularis	<2.4	Internodal Cells: 399.1 (±67.2) Corticating Cells: 80.8 (±15.9)
Cladophora glomerata	<2.4	At Mid-Cell: 75.6 (±10.2) At Cross-Walls: 37.5 (±6.0)
Bryopsis plumosa	<2.4 - 4.8	193.3 (±56.8)

Table 2. Sugar analysis of cell-wall materials. Percentages of sample mass provided by each type of monosaccharide are followed parenthetically by standard deviations. Percentages are means of duplicate analyses for each material. The percentages of total sample mass accounted for by the six monosaccharides are listed in the final column.

Cell-Wall Material	Arabinose	Fucose	Galactose	Glucose	Mannose	Xylose	Total %
Softwood Pulp	----	----	----	94.0 (±5.10)	7.6 (±0.28)	3.9 (±0.14)	101.8
Chara globularis	0.1 (±0.00)	4.6 (±0.42)	0.4 (±0.14)	78.1 (±3.68)	7.4 (±0.99)	9.4 (±0.85)	100.0
Cladophora glomerata	1.5 (±0.28)	----	3.0 (±1.13)	85.2 (±2.69)	----	4.8 (±0.42)	94.5
Bryopsis plumosa	0.5 (±0.71)	----	0.25(±0.21)	32.7 (±2.83)	1.15 (±1.06)	71.5 (±0.85)	106.1

Table 3. Densities of microcrystalline cellulose and various types of cell-wall materials, as determined by settlement in a density-gradient column. Values listed are means of triplicate analyses.

MATERIAL	DENSITY (g/cm^3)
Avicel™(microcrystalline cellulose)	1.548
Softwood Pulp	1.545
Chara globularis	1.524
Cladophora glomerata	1.561
Bryopsis plumosa	1.479

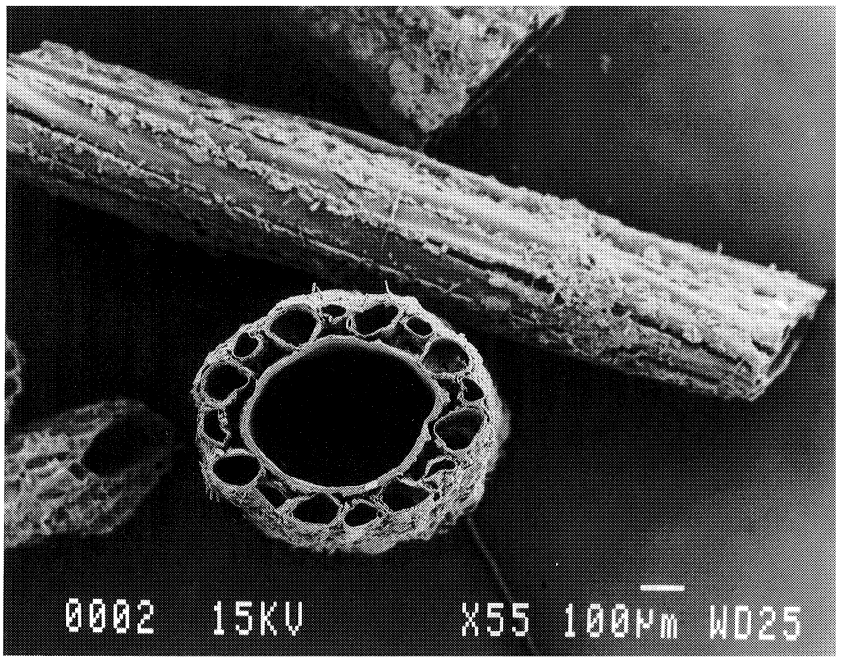

Figure 1. S.E.M. micrograph of sections cut from the main axis of a *Chara* thallus prior to decalcification and alkali boiling. Included are a section of main axis in longitudinal view, showing fluted outer surface of calcified corticating cells, and a second section in end view, showing a large internodal cell surrounded by 16 corticating cells of markedly smaller diameters. X55.

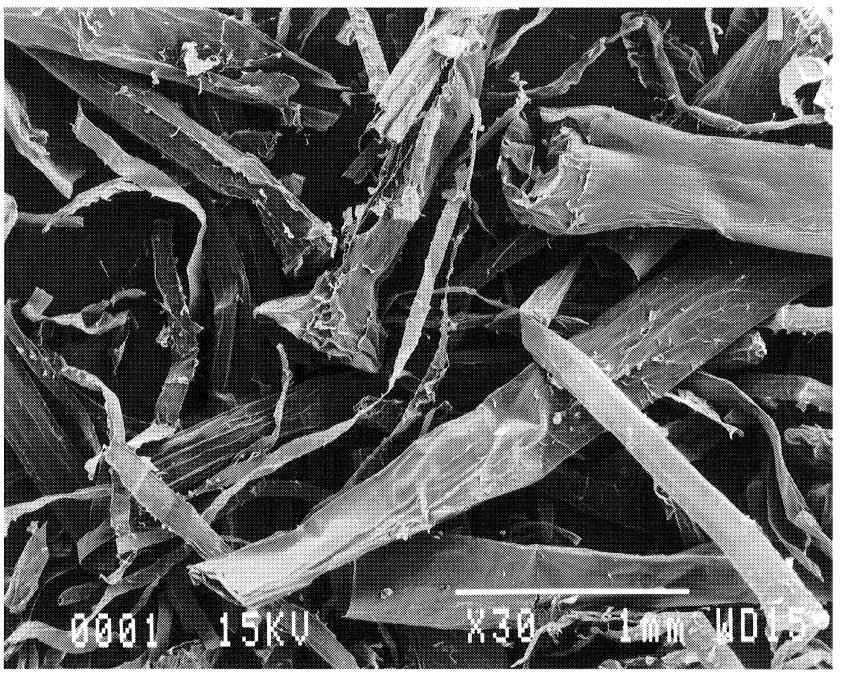

Figure 2. S.E.M. micrograph of units of *Chara* cell-wall materials following chemical treatment, which separated thalli into walls from individual cells. Note that units may be grouped into two broad ranges of diameter and occasionally possess flared ends. X30.

Figure 3. S.E.M. micrograph of processed *Cladophora* cell-wall materials, illustrating preserved, uniseriate form of filamentous construction. Occasional branches may be observed. X30.

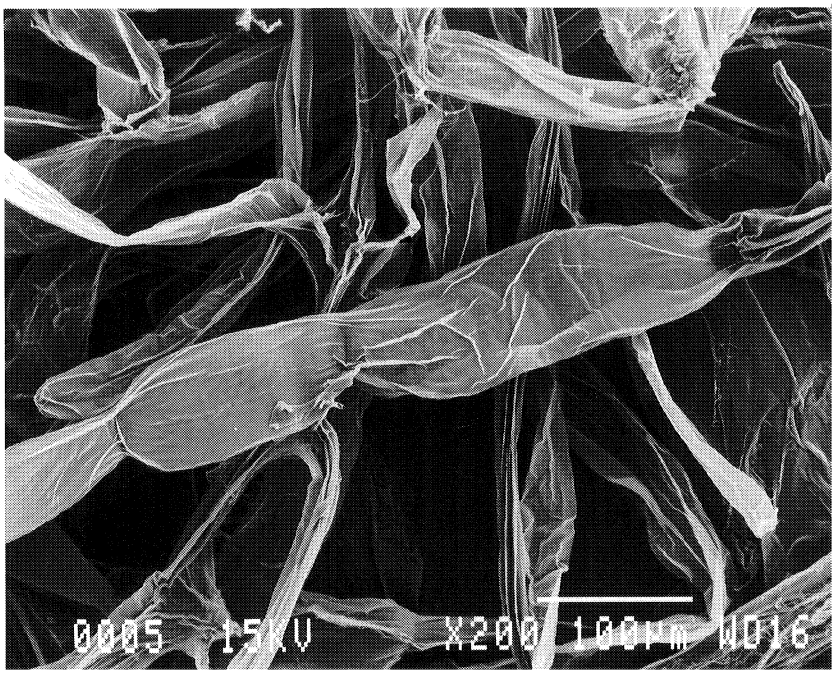

Figure 4. S.E.M. close-up of *Cladophora* material, showing cross-walls that separate individual cells within the filament. Note constriction of filament at cross-walls. X200.

Figure 5. S.E.M. microgrpah of hollow, open-ended cylinders formed after the *Bryopsis* materials were treated chemically and cut to length. Widths of individual units vary, depending upon the portion of the intact thallus from which they were cut. X30.

Figure 6. S.E.M. micrograph of softwood sulfite pulp, consisting predominately of tracheids. Individual cells in this pulp were uncut and, thus, maintained tapered ends. X30.

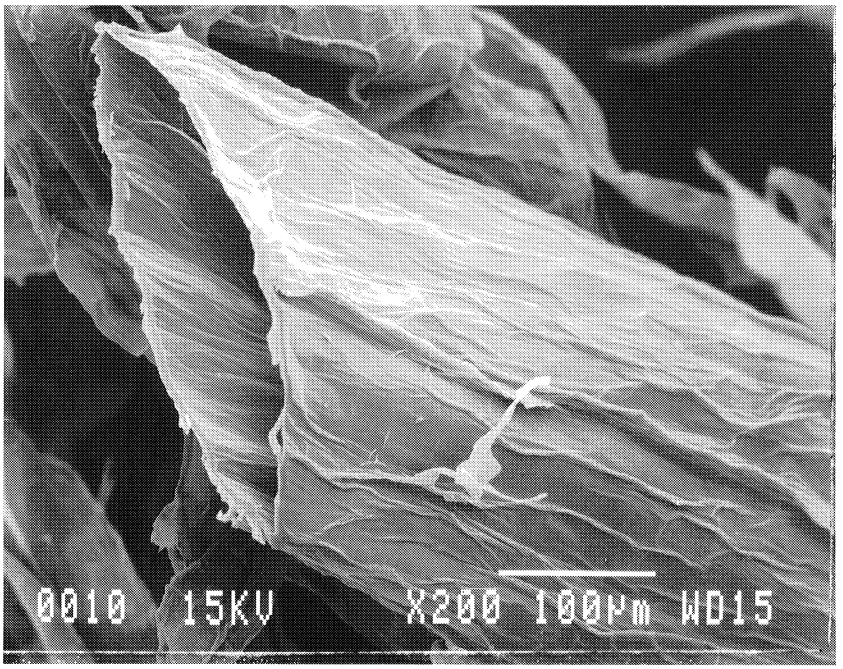

Figure 7. S.E.M. micrograph of cut end of processed *Chara* internodal cell. X200.

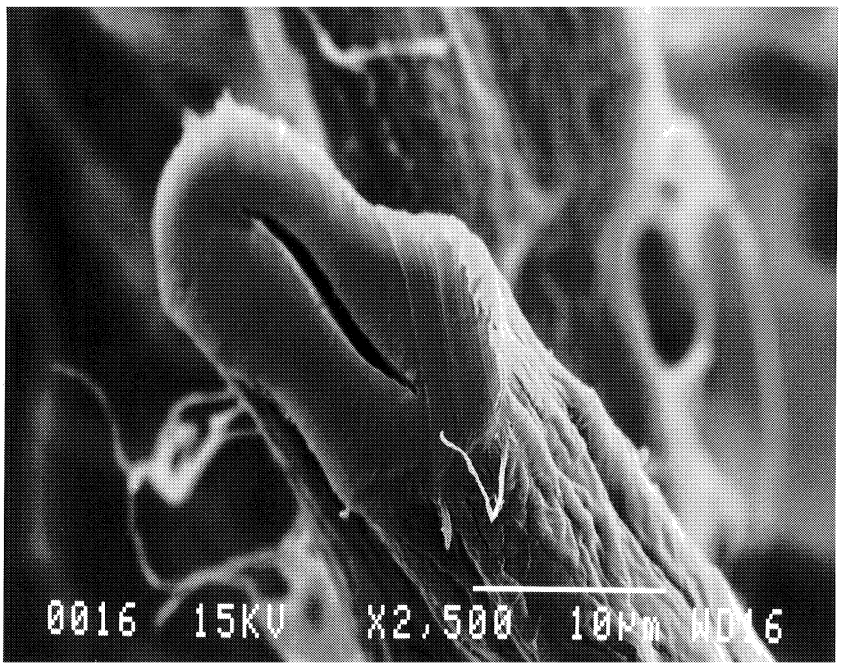

Figure 8. S.E.M. micrograph of cut end of softwood-pulp tracheid. X2500.

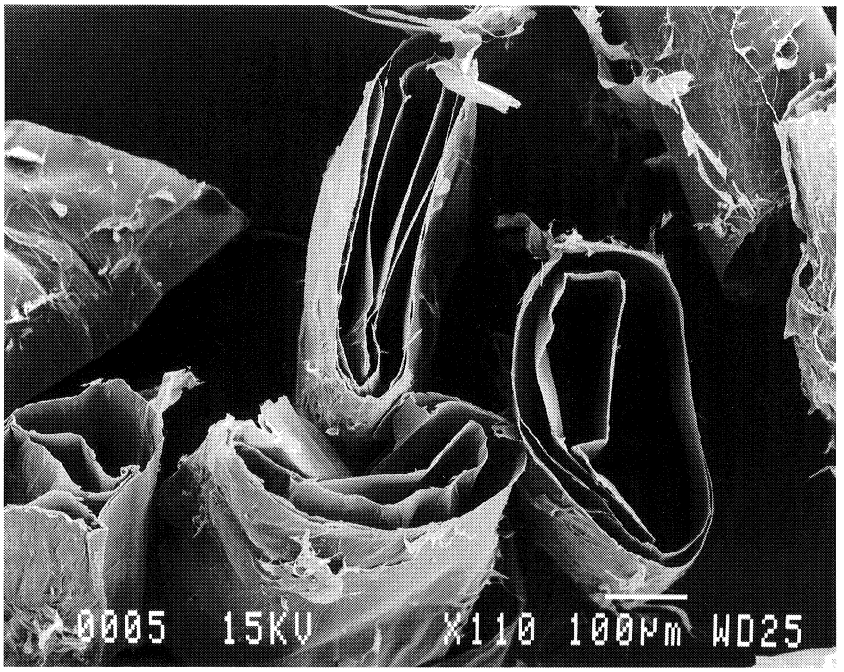

Figure 9. S.E.M. micrograph of end views of processed, mature *Chara* internodal cells, sectioned to show separation of cell walls into several layers of lamellae. X110.

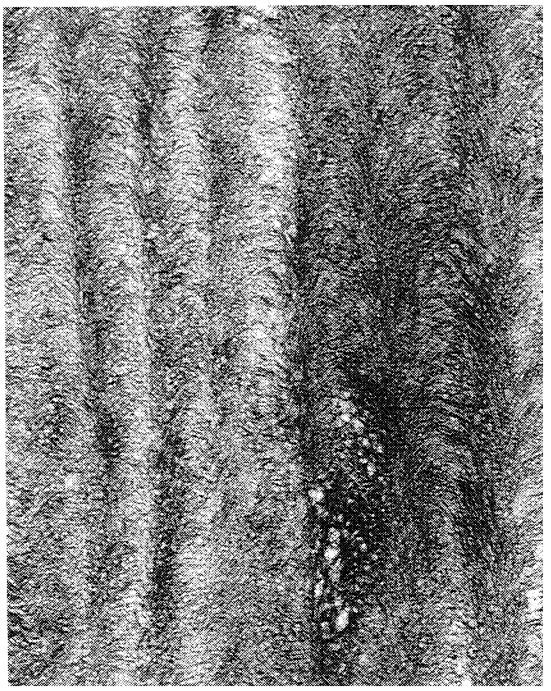

Figure 10. T.E.M. micrograph of an obliquely cut section of internodal cell wall from a *Chara globularis* shoot-tip. Area of this micrograph lay within the inner-most half of the cell wall. Note 8-10 layers of arced striations, usually interpreted as cellulose microfibrils organized into helicoidal constructions. Striations grow less pronounced toward the right of the micrograph, in the direction of the outer-most, oldest portion of the cell wall. Scale: 1mm = 25nm

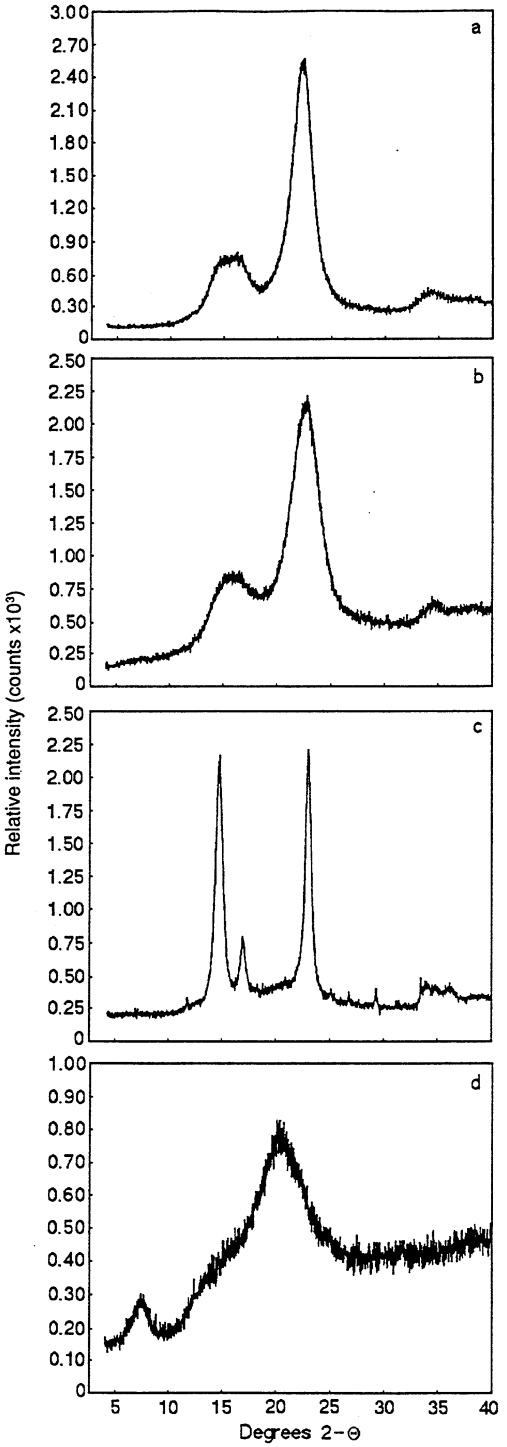

Figure 11. X-ray diffraction patterns of a) softwood sulfite pulp and of cell-wall materials isolated from b) *Chara*, c) *Cladophora*, and d) *Bryopsis*. Intensity (counts x 10^3) plotted against peak position (°two-theta). Patterns in a), b), and c) indicate presence of Cellulose I allomorph, with peaks at *ca.* 14.5, 16.0, and 22.5° two-theta corresponding to the 110, 110, and 020 planes of diffraction, respectively. The broad 110 and 110 peaks overlap in both a) and b), but high degree of crystallinity in c) allows complete separation. Pattern in d) corresponds to that of β-1,3-xylan.

Figure Legends

Figures 1-9. S.E.M. micrographs of cell-wall materials. All processed algal materials cut to lengths of *ca.* 4 mm. To aid width comparisons, a micrograph of each type of cell-wall material was taken at 30-X magnification (Figs. 2, 3, and 6). 1) Sections cut from the main axis of a *Chara* thallus prior to decalcification and alkali boiling. Included are a section of main axis in longitudinal view, showing fluted outer surface of calcified corticating cells, and a second section in end view, showing a large internodal cell surrounded by 16 corticating cells of markedly smaller diameters. 2) Units of *Chara* cell-wall materials following chemical treatment, which separated thalli into walls from individual cells. Note that units may be grouped into two broad ranges of diameter and occasionally possess flared ends. 3) Processed *Cladophora* cell-wall materials, illustrating preserved, uniseriate form of filamentous construction. Occasional branches may be observed. 4) Close-up of *Cladophora* material, showing cross-walls that separate individual cells within the filament. Note constriction of filament at cross-walls. 5) Hollow, open-ended cylinders formed after the *Bryopsis* materials were treated chemically and cut to length. Widths of individual units vary, depending upon the portion of the intact thallus from which they were cut. 6) Softwood sulfite pulp, consisting predominately of tracheids. Individual cells in this pulp were uncut and, thus, maintained tapered ends. 7) & 8) Cut ends of processed *Chara* internodal cell and softwood-pulp tracheid, respectively. Note contrasting thicknesses of cell walls. 9) End views of processed, mature *Chara* internodal cells, sectioned to show separation of cell walls into several layers of lamellae.